Acta Zootaxonomica Sinica, 30 (2): 314 - 319 (Apr., 2005) ISSN 1000-0739
动物分类学报

DESCRIPTION OF A NEW SPECIES (NEMATODA, APHELENCHOIDIDAE) ISOLATED FROM WOOD PACKAGING MATERIAL

WANG Jin-Cheng, YU Ben-Yuan, LIN Mao-Song
Plant Pathology Department of Nanjing Agricultural University, Nanjing 210095, China; E-mail: goldcitywang@yahoo.com.cn

Abstract Bursaphelenchus curvicaudatus sp. nov. is described and illustrated. Specimens were extracted from the intercepted packaging wood from Lianyungang Port, Lianyungang City, China. Bursaphelenchus curvicaudatus sp. nov. is characterized by relatively long body lengths (female 767-960 μm; male 663-831 μm), short stylets (female 13.9-17.4 μm; male 13.9-16.5 μm) with weak basal thickening, small spicules (16.5-21.6 μm) with obscure cucullus, distinguishingly bent female tails and six male caudal papillae. Lots of features such as spicule shape, female tail shape, body length supported Bursaphelenchus curvicaudatus sp. nov. distinguishes from B. hofmanni, B. abietinus, B. fungivorus, B. hellenicus, B. hylobianum, B. rainulfi, B. eggersi and B. corneolus. The PCR-ITS-RFLP pattern also provided further evidence that this isolate is a new species.

Key words Nematoda, Aphelenchoididae, Bursaphelenchus, new species, ITS-RFLP pattern, packaging wood.

Up to now, there are about 60 species under genus Bursaphelenchus Fuchs (1937) described (Yin, 1988; Braasch, 2001; Kolossova, 1998; Kanzaki, 2000; Braasch and Braasch-Bidasak, 2002; Braasch and Burgermeister, 2002). Due to the devastating pathogenicity of Bursaphelenchus xylophilus to conifer, nematodes in genus Bursaphelenchus have been studied worldwide. However, to our knowledge, most of Bursaphelenchus species merely inhabit in dead, dying or weak host trees and unrelated to parasitism.

In 2001, a piece of packaging wood, intercepted in Lianyungang port, China from a Mexican cargo ship, was sent to our lab for confirmation of pine wood nematode (PWN) existence. However, no PWN but a strange species was found. In this paper we gave a description to the species despite little information on its biology, pathogenicity, embrology was known.

1 Materials and Methods

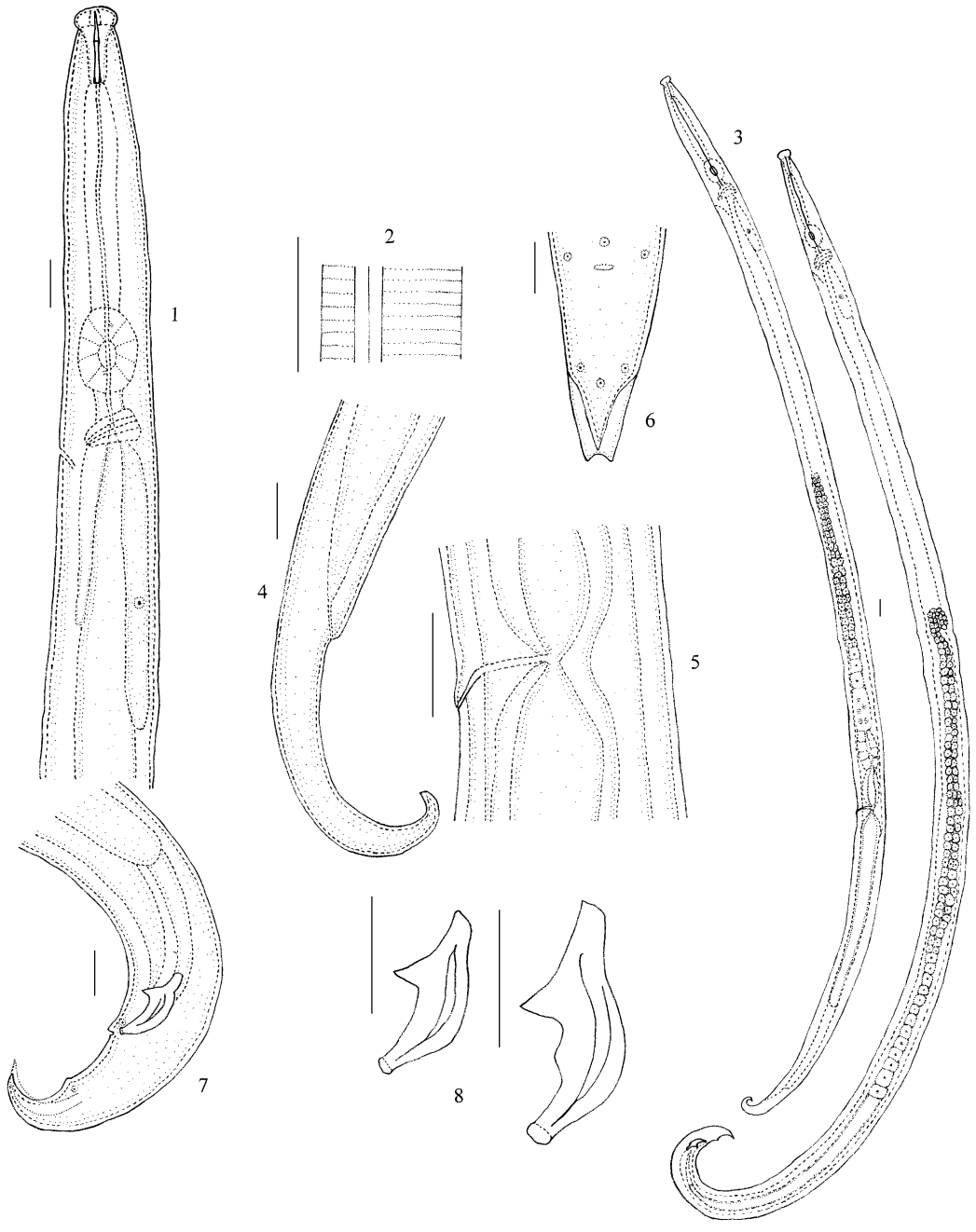
Nematodes were isolated by Baermann funnel method from the cut slices of the packaging wood. Then ca 100 nematodes with similar shape were picked into the mycelium of Botritis cinerea on the PDA medium for further culture at 25 ℃. In 2002, we further purified the isolate by just breeding one pregnant female in a petri dish with fresh mycelium of Botritis cinerea. Then purified specimens were killed by incubating at 65 ℃ for 90 sec., fixed in TAF for further investigation. All the specimens used for morphological measurement were mounted in permanent slides.

The PCR-rDNA-RFLP analysis as auxiliary identification technique was applied. The method of DNA extraction refers to the published protocol with some modification (Subbotin et al., 2000).

Firstly, 2 or 3 nematodes were picked into 15 μl ddH_2O and each was cut into 3 segments using No. 3 scalpel. Then all the nematode segments with 12 μl ddH_2O were transferred into 200 μl PCR tubes. 1.5 μl 10 × PCR reaction buffer and 1.5 μl proteinase K (1 000 μg/ml) were added into each PCR tube to complete total volume of 15 μl. The tubes were frozen at - 80 ℃ for at least 30 min. At last, the tubes were incubated at 65 ℃ for 60 min and at 95 ℃ for 10 min.

The PCR reaction system (50μl) contained 0.6 μmol/L of each primer, 2 mmol/L of $MgCl_2$, 0.1 mmol/L dNTPs and 2 units of Taq polymerase (Hoyer et al., 1998). The forward primer is 5′-CGTAACAAGGTAGCTGTAG-3′ (Ferris et al., 1993) and the reverse primer is 5′-TTTCACTCGCCGTTACTAAGG-3′ (Vrain, 1993). The PCR reaction was carried out as the following procedure: 94 ℃ for 2.5 min; 40 cycles of 94 ℃ for 40 sec, 55 ℃ for 40 sec, 72 ℃ for 90 sec; 72 ℃ for 5 min. 5 μl of the PCR amplified product was used for electrophoresis to check if the concentration of the amplification product is enough for the enzyme-cutting. Suitable tube of amplification product was chosen and digested by restriction endonuclease Rsa Ⅰ, Hae Ⅲ, Msp Ⅰ, Hinf Ⅰ and

The project was supported by the "863 Project" programme of Ministry of Science and Technology, China.
Received 6 Sep. 2004, accepted 20 Dec. 2004.



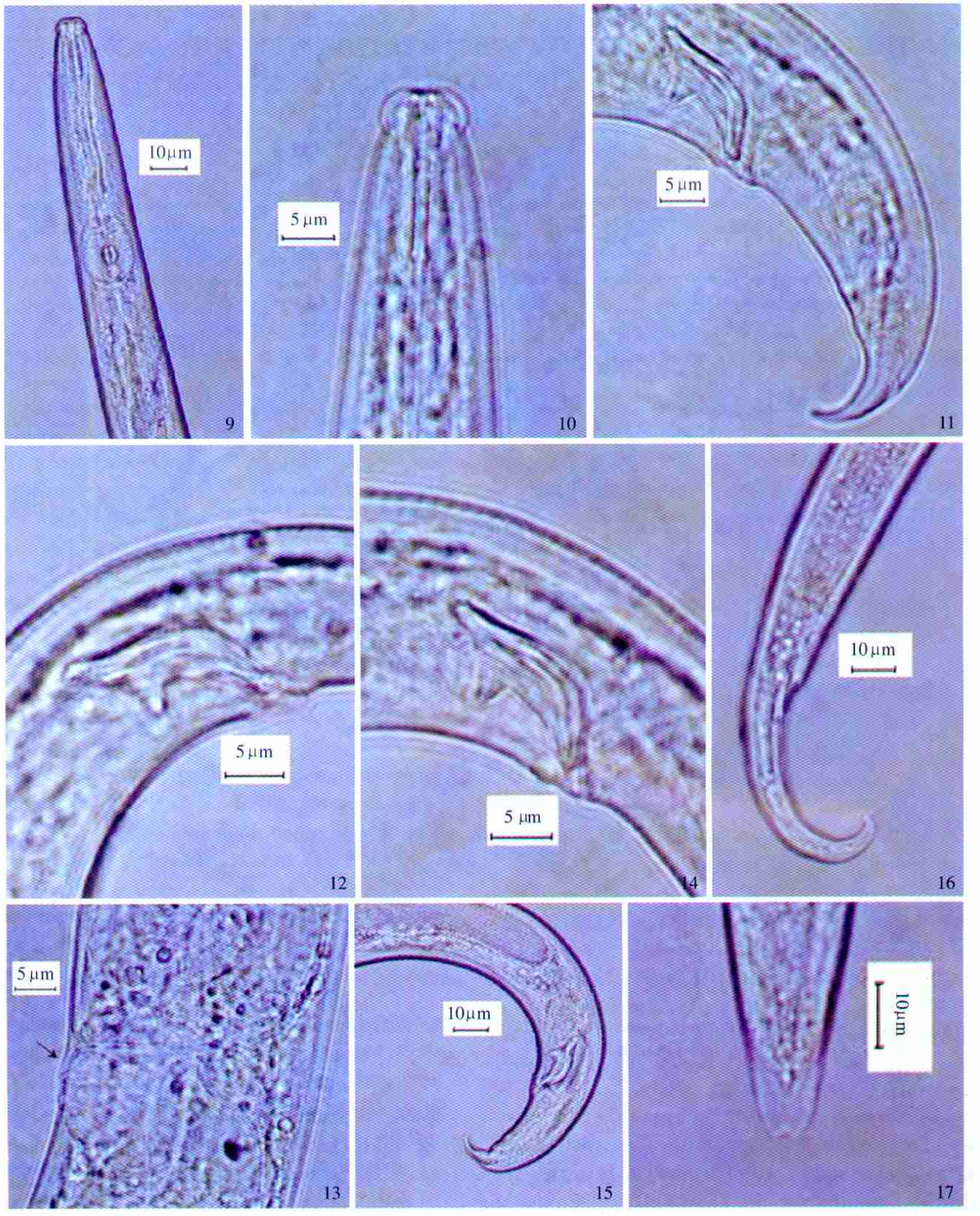
Figs. 1-8. Bursaphelenchus curvicaudatus sp. nov. 1. Anterior part. 2. Lateral lines. 3. Whole bodies of female and male. 4. Female tail. 5. Vulval flap. 6. Male tail (ventral view). 7. Male tail (lateral view). 8. Spicules. Scale bars = 10 μm.

Alu I with each enzyme cutting 7 μl of the PCR product. Enzyme-cutting fragments were resolved on 2 % agrose gel and stained with 1 % ethidium bromide. The details of constructing the ITS-RFLP profile refer to the published protocol (Hoyer et al., 1998; Braasch et al., 1999)

2 Result

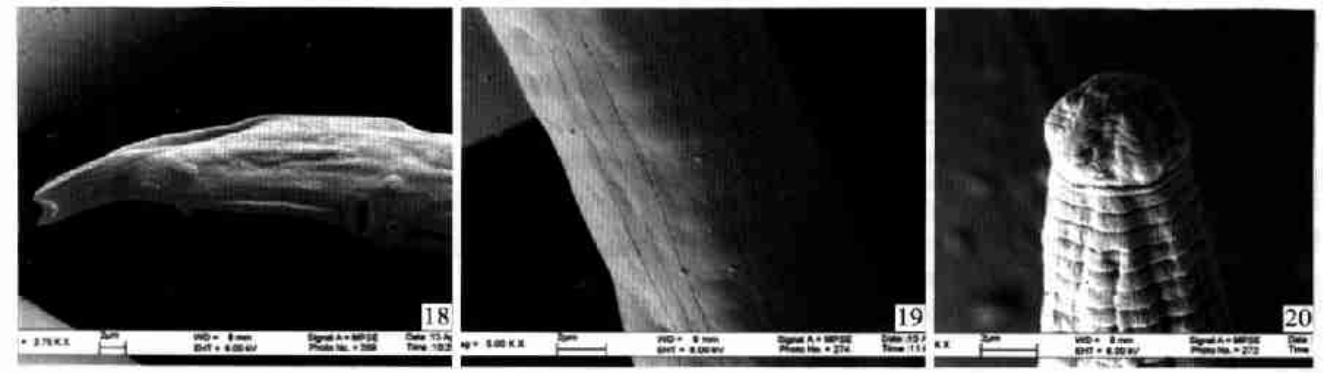
Bursaphelenchus curvicaudatus sp. nov. (Figs. 1-20)

Description (unit: μm, Table 2). Female. Body slender, cylindrical, slightly ventrally curved when heat-killed. Lip region high with six lips of apparently equal size, anteriorly flat, offset with body by distinct constriction; labial annules present (Fig. 20); the circular oral aperture surrounded by a nearly rectangular depression. Stylet 13.9-17.4 μm long, slender, with weak basal thickening. Stylet cone less than half of total stylet length. Median bulb oval, occupying ca 3/4 of body diameter, with distinct valve situated centrally. Excretory pore very faint, ca 1.0-1.5 body dia-



Figs. 9-17. Light microscope observations of Bursaphelenchus curvicaudatus sp. nov. 9. Anterior part. 10. Head. 11, 12, 14. Spicules. 13. Female vulval flap. 15. Male tail (heat-killed). 16. Female tail (heat-killed). 17. Bursa.

meters behind median bulb. Dorsal oesophageal gland strong, ca 3 to 4 body diameters long, overlapping intestine. Reproductive system single and prodelphic. Gonad outstretched, developing oocytes in multiple files (2 to 3 files) except at the posterior end. Vulval flap short and small. Postuterine sac beyond 1/2 of the



Figs. 18-20. Scanning electronic microscope observations of Bursaphelenchus curvicaudatus sp. nov. 18. Male tail. 19. Lateral field. 20. Head. Scale bars = 2 μm.

Table 1. Restriction fragments of the amplified PCR product of the new species B. curvicaudatus sp. nov.

PCR production	Restriction fragments				
	Rsa Ⅰ	Hae Ⅲ	Msp Ⅰ	Hinf Ⅰ	Alu Ⅰ
1 150	500	550	380	620	920
	430	390	260	290	230
	220	220	190	240	

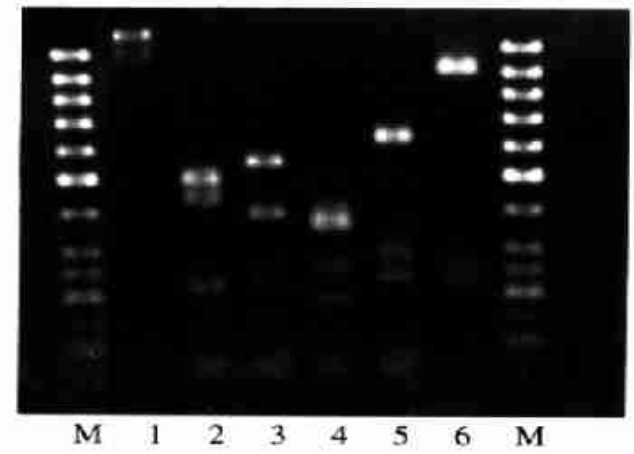


Fig. 21. The ITS-RFLP pattern of Bursaphelenchus curvicaudatus sp. nov. Lane M: maker. Lane 1: PCR product of ITS regions of Bursaphelenchus curvicaudatus sp. nov. Lane 2, 3, 4, 5, 6: the enzyme-cutting fragments of PCR product digested with Rsa Ⅰ, Hae Ⅲ, Msp Ⅰ, Hinf Ⅰ, Alu Ⅰ respectively

vulva-anus distance. Tail sharply concoid with terminus distinctly curved ventrally. Three lateral lines (i. e. 2 ridges) in the midbody (Figs. 2, 19).

Male. Body, J-shaped after heat-killed, anterior region similar to the females. Lateral region with three incisures in the midbody. Testis reflexed or not, extending for ca 50 % of body length. Spicule is small, 16.5-21.6 μm long, with a triangle rostrum and a high condylus. Cucullus hardly visible unless observed under 1 000 × magnification. Tail claw-like, terminus pointed, the distal edge of the terminal bursa in crescent shape. Six caudal papillae present. One pair of adanal ones ca. 1 μm above the cloaca, one preanal papilla on the ventral midline ca. 0.3 μm higher than the adnal pair, and three postanal papillae arranged in triangle form, i. e. one subventral pair located at the proximal end of the bursa, another midventral one about 3 μm lower (Figs. 6, 18).

Diagnosis and relationship. Bursaphelenchus curvicaudatus sp. nov. is characteristic by its small spicule with very weak cucullus, big rostrum and tall condylus, small female vulval flap, slim and distinctly ventrally curved female tail. According to the shape of the male spicule, the female tail and some other features, B. curvicaudatus resembles B. hofmanni (Braasch, 1998), B. abietinus (Braasch & Schmatzenhofer, 2000), B. fungivorus (Franklin & Hooper, 1962), B. hellenicus (Skarmoutsos, Braasch & Michalopoulou, 1998), B. hylobianum (Korenchenko, 1980), B. rainulfi (Braasch & Braasch-Bidasak, 2002), B. corneolus (Massey, 1966) and B. eggersi (Rühm, 1956).

B. curvicaudatus sp. nov. distinguished from B. hellenicus by its bigger "a" ratio (female: 30-45 μm vs 22-36 μm; male: 32-40 μm vs 22-38 μm) and bigger spicules (16.5-21.6 vs 11.5-17.5 v). The female tails of B. hellenicus look almost straight, which also differ from the distinctly curved ones of the new species. The morphometrics of B. fungivorus are very similar to the new species, whereas, its spicules lack cucullus, its incisures in the midbody are more (4 vs 3). The spicules of B. eggersi are relatively longer (18-24 μm vs 17-22 μm) with no cucullus, and the ventral sides are almost straight distinguishing the smoothly bent ones of the new species. The spicules and female tails of B. hofmanni is morphologically almost the same as those of the new species, and yet its stylets are shorter (female: 11-14 μm vs 14-17 μm;

male: 11-13 μm vs 13.9-16.5 μm), its "a" ratio is much smaller (female: 23-31 μm vs 30-45 μm; male: 22-31 μm vs 32-40 μm) and its spicules are shorter (11-17 vs 17-22 μm). The measurements of B. abietinus are distinctly from the new species, meanwhile, B. abietinus has two lateral lines in contrast to three ones of the new species. B. rainulfi has a extremely similar spicule and female tail to the new species, and yet the distal edge of the bursa of the new species is cresent which differs from the irregular triangle shape of B. rainulfi; the incisures of B. rainulfi are two, less than the new species. B. hylobianum has two incisures. In addition, the terminal bursa of B. hylobianum is three or four-pointed in shape different from the crescent form of the new species. Comparing with the new species, B. corneolus is much shorter (female: 650-700 μm vs 767-960 μm; male: 570-700 μm vs 663-831 μm) and has smaller spicules (13 μm vs 17-22 μm), smaller stylets (female: 12 μm vs 13.9-17.4 μm; male: 12 μm vs 14-17 μm), and the stylets of B. corneolus is devoid of basal swellings. More details on the morphometrics of the published species similar to the new species are given in the paper (Table 3).

The molecular analysis also showed that the rDNA-PCR-RFLP pattern of the new species (Fig. 21) differs from those of B. abietinus, B. hellenicus, B. hylobianum and B. rainulfi (Braasch and Burgermeister, 2002), and from B. fungivorus, B. hofmanni and B. eggersi (Braasch et al., 1999).

Table 2. Measurements of Bursaphelenchus curvicaudatus sp. nov. (unit: μm)

	Females		Males	
	Holotype	paratypes		Paratypes
n	1	20	1	20
L	779	894 ±54 (767-960)	830	750 ±52 (663-831)
a	36.6	35.4 ±4.0 (30.2-45.1)	36.7	35.0 ±2.4 (31.5-40.2)
b	10.7	11.2 ±0.7 (10.1-12.6)	8.4	9.2 ±0.9 (8.0-10.4)
c	15.6	17.2 ±1.6 (15.0-20.2)	21.4	19.2 ±1.5 (16.7-22.9)
V	74.5	71.9 ±1.5 (69.7-74.8)	—	—
st	15.2	15.1 ±0.8 (13.9-17.4)	14.4	15.5 ±0.9 (13.9-16.5)
sp	—	—	18.1	18.9 ±1.6 (16.5-21.6)

St: stylet; Sp: spicule.

Type host and locality. The intercepted packaging wood from a Mexican cargo ship, which can only be identified as conifer.

Type material. Now all the permanent slides are deposited in the Nematology Laboratory of Nanjing Agricultural University, Nanjing, China.

Table 3. Key morphometrics of Bursaphelenchus spp. morphologically similar to the new species. [data (unit: μm) from published papers: Braasch, **2002**b, Yin et al., **1988**]

		L	Stylet	a	c	Spicule
B. curvicaudatus *	F	767-960	13.9-17.4	30.2-45.1	15.0-20.2	-
	M	663-831	13.9-16.5	31.5-40.2	16.7-22.9	16.5-21.6
B. hellenicus	F	680-920	13-17	22-36	17-31	-
	M	640-820	13-17	22-38	19-30	11.5-17.5
B. hofmanni	F	450-630	11-14	23-31	13-21	-
	M	489-610	11-13	22-31	16-31	11-17
B. eggersi	F	850-1122	14-18	37-39	20	-
	M	450-990	14-16	21-31	19-28	18-24
B. abietinus	F	540-710	11-14	23-32	16-26	-
	M	530-670	11-13	24-34	18-30	11-14
B. rainulfi	F	525-750	11-14	23-40	15-25	-
	M	475-750	11-13	25-44	19-38	12-15
B. hylobianum	F	541-965	14-17	21-36	14-25	-
	M	473-846	14-16	25-35	15-26	18-22
B. fungivorus	F	610-1160	14-18	26-44	10-17	-
	M	570-1030	13-18	26-45	18-32	15-23
B. corneolus	F	650-700	12	29	18	-
	M	570-700	12	35	18	13

F: female; M: male. * spicules measured along the middle line.

3 Discussion

Although the new species was isolated from the packaging material from the Mexican cargo ship, it's thoughtless to determine that the new species originates from Mexico. Theoretically, the DNA quantity of only one nematode is enough for the PCR-ITS-RFLP analysis. However, in our studies we couldn't obtain the ideal PCR-ITS-RFLP patterns. In fact, 2 or 3 nematodes are necessary. Sometimes, a faint DNA band (Fig. 21) following the main PCR product (1 150 bp)

could be visualized, which we think is the result of nonspecific amplification. Anyway, it doesn't affect the ITS-RFLP pattern of the new species, which serves as an identification label. Generally, the male caudal papillae in genus Bursaphelenchus are seven, however, Bursaphelenchus curvicaudatus is different by displaying six ones, which is another special feature for the new species.

Acknowledgements The first author is grateful for the help of MA Yi-Gui, Tianjin Entry-exit Inspection and Quarantine Bureau, China, during preparation of the manuscript.

REFERENCES

Braasch, H. and Burgerneister, W. 2002a. Bursaphelenchus rainulfi sp. n. (Nematoda: Parasitaphelenchidae), first records of Bursaphelenchus Fuchs, 1937 from Malaysia. Nematology, 4: 971-978.

Braasch, H. and Braasch-Bidasak, R. 2002b. First record of the genus Bursaphelenchus Fuchs, 1937 in Thailand and description of B. thailandae sp. n. (Nematoda: Parasitaphelenchidae). Nematology, 4: 853-863.

Braasch, H. 2001. Bursaphelenchus species in conifers in Europe: distribution and morphological relationships. OEPP/ EPPO Bulletin, 31: 127-142.

Braasch, H., Metge, K. and Burgermeister, W. 1999. Bursaphelenchus species (Nematoda: Parasitaphelenchidae) found in coniferous trees in Germany and their ITS-RFLP patterns. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 51: 312-320.

Braasch, H., Tomiczek, Ch., Metge, K., Hoyer, U., Burgermeister, W., Wulfert, I. and Schoenfeld, U. 2001. Records of Bursaphelenchus spp. (Nematoda: Parasitaphelenchidae) in coniferous timber imported from the Asian part of Russia. Forest Pathology, 31: 129-140.

Ferris, V. R., Ferris, J. M. and Faghihi, J. 1993. Variations in spacer ribosomal DNA in some cyst forming species of plant parasitic nematodes. Fundamental and Applied Nematology, 16: 177-184.

Fuchs, A. G. 1937. Neue parasitische und halbparasitische Nematoden bei Borkenkäfern und einige andere Nematoden. I. Teil Die Parasiten der Waldgärtner Myelophilus piniperda L. und minor Hartig und die Genera Rhabditis Dujardin, 1845 und Aphelenchus Bastian, 1865. Zoologische Jahrbücher Abteilung Für Systematik Oekologie und Geographie der Tiere, 70: 291-380.

Hoyer, V. U., Burgermeister, W. and Braasch, H. 1998. Identification of Bursaphelenchus species (Nematoda: Aphelenchoididae) on the basis of amplified ribosomal DNA (ITS-RFLP). Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, 50: 273-277.

Kanzaki, N., Tsuda, K. and Futai, K. 2000. Description of Bursaphelenchus conicaudatus n. sp. (Nematoda: Aphelenchoididae), isolated from the yellow-spotted longicorn beetle, Psacothea hilaris (Coleoptera: Cerambycidae) and fig trees, Ficus carica. Nematology, 2: 165-168.

Kolossova, N. V. 1998. Bursaphelenchus eroshenkii sp. n. (Nematoda: Aphelenchoididae) from the Russian Far East, with a key to some species of Bursaphelenchus Fuchs, 1937. Russian Journal of Nematology, 6: 161-164.

Subbotin, S. A., Waeyenberge, L. and Mones, M. 2000. Identification of cyst forming nematodes of the genus Heterodera (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLP. Nematology, 2: 153-164.

Vrain, T. C. 1993. Restriction fragment length polymorphism separates species of Xiphinema amerianum group. Journal of Nematology, 25: 361-364.

Yin, K., Fang, Y. and Tarjan, A. C. 1988. A key to species in the genus Bursaphelenchus with a description of Bursaphelenchus hunanensis sp. n. (Nematoda: Aphelenchoididae) found in pine wood in Hunan Province, China. Proceedings Helminthological Society of Washington, 55: 1-11.

木质包装材料中线虫一新种 (线虫门, 滑刃科) 记述

王金成　余本渊　林茂松

南京农业大学植物病理系　南京　210095; E-mail: goldcitywang@yahoo.com.cn

摘　要　记述了伞滑刃属线虫 1 新种, 即弯尾伞滑刃线虫 Bursaphelenchus curvicaudatus sp. nov.。线虫样本自中国连云港口岸截获木质包装材料中分离获得。新种体长相对较长 (雌虫 767～960 μm; 雄虫 663～831 μm), 口针较短 (雌虫 13.9～17.4 μm; 雄虫 13.9～16.5 μm), 口针基部略微加厚, 交合刺小 (16.5～21.6 μm), 末端盘状结构不明显, 雌虫尾明显向腹面弯曲。另外, 新种雄虫有 6 个尾乳突, 与以前报道有 7 个尾乳突不同。交合刺形状、雌虫尾的形状及体长等特征能将新种与 B. hofmanni、B. abietinus、B. fungivorus、B. hellenicus、B. hylobianum、B. rainulfi 、B. eggersi 以及 B. corneolus 区分开来。新种特有的限制性酶切图谱 (PCR-ITS-RFLP 图谱) 是该分离物为 1 新种的分子证据。

关键词　线虫门, 滑刃科, 伞滑刃属, 新种, ITS-RFLP 图谱, 包装材料.

中图分类号　Q959.17